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Systematics of *Trimeresurus popeiorum* Smith, 1937 with a revised molecular phylogeny of Asian pitvipers of the genus *Trimeresurus* Lacépède, 1804 *sensu lato*

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Abstract

The pit viper snake genus *Trimeresurus* Lacépède, 1804 *sensu lato*, is a diverse group of nocturnal serpents comprising over 61 species. The genus is morphologically heterogeneous and has been divided into several subgenera. We present an updated phylogeny of Asian pitvipers and propose a revised classification. Additionally, we revise the taxonomy of *T. popeiorum* Smith, 1937 and propose taxonomic changes with support from molecular and morphological data. We restrict *T. popeiorum sensu stricto* to northeastern India, Bangladesh, southern China, and northern Myanmar; populations beyond these areas require further assessment. We also synonymize *T. yingjiangensis* Chen et al., 2019 with *T. popeiorum* based on overlapping morphological characters, molecular data, and distribution. The findings shed new light on the taxonomy of *T. popeiorum*, warranting the need for assessing the population of *T. popeiorum* from southeast Asia.

Key Words

Asia, molecular phylogeny, *Popeia*, synonymy, subgenus, systematics, Viperidae

Introduction

The pit viper snake genus *Trimeresurus* Lacépède, 1804 *sensu lato*, is a diverse group of nocturnal serpents (Smith 1943; Gumprecht et al. 2004) with 61 known species distributed throughout South and Southeast Asia (Chen et al. 2019, 2021; Mirza et al. 2020; Uetz and Hošek 2023). Molecular data have shown that the genus is paraphyletic (Pyron et al. 2013; Zaher et al. 2019). Still, its division into distinct genera (Malhotra and Thorpe 2004a) has not been accepted due to nomenclatural issues (David et al. 2011). The subgeneric taxonomy proposed by David et al. (2011) has likewise not been followed in a unified manner by subsequent studies. The several new pitviper species described since 2004 have generally been referred to as *Trimeresurus sensu lato* (Whitaker and Captain 2004; Captain et al. 2019), while others use the putative subgeneric names as distinct genera (Creer et al. 2006; Guo and Wang 2011; Malhotra et al. 2011; Guo et al. 2015; Li

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et al. 2020). Still, other researchers comment on the subgeneric species placement but refrain from using the subgeneric names (Mirza et al. 2020; Rathee et al. 2022). Taxonomy and nomenclature aside, the morphologically almost indistinguishable and highly variable members of *Trimeresusus* serve as an ideal model for studying cryptic species and their diversification (Malhotra and Thorpe 2004a; Sanders et al. 2004; Guo et al. 2015; Zhu et al. 2016; Mulcahy et al. 2017). We herein follow the taxonomy suggested by Malhotra and Thorpe (2004a), incorporating the recommendations by David et al. (2011).

Several researchers have explored the phylogenetic relationships within *Trimeresurus sensu lato*, and the relationships have been relatively well studied (Malhotra and Thorpe 2000, 2004a; Sanders et al. 2004; Creer et al. 2006; Pyron et al. 2013; Guo et al. 2015; Figueroa et al. 2016; Zhu et al. 2016; Mulcahy et al. 2017; Zaher et al. 2019). Recent descriptions of new species provide molecular data that add additional support to established phylogenetic relationships (Captain et al. 2019; Chen et al. 2019; Mirza et al. 2020). In the present study, we re-analyze existing data by adding new molecular data to propose a revised classification proposed by earlier researchers (Malhotra and Thorpe 2004a).

Trimeresurus popeiorum Smith, 1937 is a common venomous snake distributed across parts of China, Bhutan, northeastern India, Bangladesh, Myanmar, Thailand, Malaysia, and northern Laos (Sanders et al. 2006; Mulcahy et al. 2017). This species was originally identified as '*Trimeresurus gramineus*' by Pope & Pope, (1933), but later M. A. Smith (1937) described this species as '*Trimeresurus popeiorum*' honouring C. H. Pope and S. H. Pope. Moreover, as the incorrect spelling '*popeiorum*' appeared due to a clerical mistake, in subsequent works M.A. Smith reffered to this species as to '*Trimeresurus popeorum*' (Smith, 1943), however the original spelling should be retained (David and Vogel 1996). The specimens examined by M. A. Smith originate from northeastern India, and the type locality of this species was restricted to "Khasi Hills, Assam" (now in Meghalaya), by Taylor and Eelbel (1958). Recent studies have shown considerable genetic diversity within this species complex (Mulcahy et al. 2017), and an attempt was made to refine the taxonomy of the group (Vogel et al. 2004). However, as recently as 2019, *T. yingjiangensis* – a new species in this complex was described from China (Chen et al. 2019), which hints at the presence of additional cryptic diversity. *Trimeresurus yingjiangensis* was also recently reported for northern Myanmar by Shuo et al. (2022). Herein we present morphological and molecular data for *T. popeiorum* based on freshly collected specimens from across northeastern India and Myanmar, which provide newer insights into the systematics of the group. A phylogeny of Asian pit vipers based on 82% of the known species is presented, along with a revised taxonomy.

Methods

Morphology

The study was approved by the Department of Environment, Forests and Climate Change, Government of Arunachal Pradesh and Mizoram under permit no. CWL/Gen/173/2018-19/Pt.V11/2421-33 and CWL/Gen/173/2018-19/Pt.V11/2434-43 and No.A.33011/2/99-CWLW/225, respectively. Specimens were collected by means of snake hooks in the field, photographed, and euthanized within 24 h of capture using halothane, following ethical guidelines for animal euthanasia (Underwood and Anthony 2020). Liver tissue was collected for molecular work and stored in molecular-grade ethanol before specimen fixation. Specimens were fixed in 8% buffered formalin and later stored in 70% ethanol. They have been deposited in the collection of the Bombay Natural History Society, Mumbai, Maharashtra, India (**BNHS**), the Departmental Museum of Zoology, Mizoram University, Aizawl, Mizoram, India (**MZMU**), and the research collection of the National Centre for Biological Sciences, Bangalore, Karnataka, India (**NCBS**), Zoological Museum of Moscow University, Moscow, Russia (**ZMMU**). The lecotype of *Trimeresurus popeiorum* housed at the Natural History Museum, London (**NHMUK**) was examined too.

Detailed measurements were taken with digital callipers to the nearest 0.1 mm, and those for snout-to-vent length (SVL) and tail length (TaL) were taken with a string, which was then measured using a ruler. The style of the description follows Mirza et al. (2020) with some modifications. Ventral scales (V) were counted as directed by Dowling (1951). The number of cephalic scales (CEP) was counted in a straight line between supraoculars; longitudinal cephalic scales (LCS) were counted from the posterior border of the internasals to the point on the neck identified by the dorsal scale row that corresponds to the first ventral scale. Abbreviations used in the description include TL (total length), HL (head length, measured from snout tip to angle of the jaw), VED (vertical extent of the eye), DEYE (eye diameter, measured between the edges of scales surrounding the eye), DEL (distance between the lower eye margin and the lower edge of a supralabial). Morphological data from the literature were largely derived from Gumprecht et al. (2004).

Micro-CT scans were generated for a male specimen using a Bruker Skyscan 1272 (Bruker BioSpin Corporation, Billerica, Massachusetts, USA). The head of the specimen was scanned for 210 min at 3 µm resolution, recording data for every 0.4° of rotation with an aluminium 1 mm filter, source voltage 65 kV, and source current 153 uA. Volume rendering was performed with the scan's CTVox (Bruker BioSpin Corporation, Billerica, Massachusetts, USA) software, and images were edited in Adobe Photoshop. Morphological characters of the skull and osteological terminology generally followed the terminology of Heatwole (2009) and Seghetti et al. (2021).

Molecular analysis

Genomic DNA was isolated from preserved tissues using QIAGEN DNeasy kits following manufacturer protocols. Molecular methods largely follow Mirza et al. (2016) and Mirza and Patel (2018). A fragment of the mitochondrial *16S* rRNA (*16S*) was amplified using primers used by Mirza et al. (2016) for samples from India and cytochrome *b* as well for two specimens from Myanmar. A 22.4-µl reaction was set up for bi-directional Polymerase Chain Reaction (PCR), containing 10 µl of Thermo Scientific DreamTaq PCR Master Mix, 10 µl of molecular grade water, 0.2 µl of each 10 µM primer, and 2 µl template DNA, carried out with an Applied Biosystems ProFlex PCR System. The thermocycle profile used for amplification was as follows: 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 45 °C for 45 sec, elongation at 72 °C for 1 mins) for 36 cycles, 72 °C for 10 min, and hold at 4 °C. The PCR product was cleaned using a QIAquick PCR Purification Kit and sequenced with an Applied Biosystems 3730 DNA Analyzer. In addition to obtaining our data for *16S*, we also obtained sequences for cytochrome *b*, *12S* rRNA, and *ND4* of *Trimeresurus* species available on GenBank for molecular phylogenetic reconstructions (see Suppl. material 1 for accession numbers of sequences generated in the present study and those used in the analysis). Sequences were concatenated using SequenceMatrix (Vaidya et al. 2011).

Taxa for molecular phylogenetics were selected based on the tree topologies recovered by Figueroa et al. (2016). Sequences were aligned in MegaX (Kumar et al. 2018) using ClustalW (Thompson and Gibson 2002) with default settings. *Azemiops feae* and *Protobothrops elegans* were used as outgroup taxa for the phylogenetic analysis following the recent results on phylogenetic relationships of caenophidian snakes (Zaher et al. 2019). The aligned dataset was subjected to analysis on the IQ-TREE online portal (Minh et al. 2020). A sequence substitution model was selected using the *auto* parameter with a provision for FreeRate heterogeneity (Suppl. material 1), and the analysis was run with an ultrafast bootstrap option for 1000 iterations to assess clade support. Furthermore, single-branch tests were performed for the approximate Bayes test. The tree was visualized and edited in FigTree (Rambaut 2012). Un-corrected p-distance was calculated for *16S* in MegaX and missing data or gaps were subjected to the pairwise deletion option.

Results

Molecular phylogenetics

Molecular data for four mitochondrial genes comprising 2391 bp were used to assess phylogenetic relationship. Results from the analysis are congruent with previous work (Malhotra and Thorpe 2004a; Sanders et al. 2004; Guo et al. 2015; Mulcahy et al. 2017; Mirza et al. 2020) but provided some additional insights (Fig. 1). The subgenus *Popeia* is represented by six major clades, as reported by Chen et al. (2019). Clade A contains *T. sabahi*, *T. barati*, *T. toba*, *T. fucatus*, and *T. buniana* however, we do not recover each of these as distinct clades and hence we propose to treat all these taxa under the name *T. sabahi* until further evidence is available for their validity. Clade B represents *T. nebularis* and Clade C consists of *T.* cf. *popeiorum* (regarded as *Trimeresurus popeiorum* previous researchers example, Chen et al. 2019) from China, Laos, Thailand, and Myanmar, whereas Clade D represents *T. phuketensis*. Clade E includes an unnamed taxon from Thailand and Myanmar earlier reported by Mulcahy et al. (2017). Clade F represents *T. popeiorum sensu stricto* and includes populations from India, northern Myanmar (Kachin and Sagaing regions) and from Yunnan Province of China. The Chinese sample of these populations includes specimens of *T. yingjiangensis*. Clade F is here identified as *Trimeresurus popeiorum sensu stricto* based on sequences from India: Darjeeling (West Bengal), Eaglenest Wildlife Sanctuary and Kamlang Wildlife Sanctuary (Arunachal Pradesh), Digboi (Assam), and Aizawl (Mizoram), and from northern Myanmar along with sequences of *Trimeresurus yingjiangensis* (China), which is here considered as a subjective junior synonym of *Trimeresurus popeiorum* based on molecular (Fig. 1) and morphological data (see Table 1 and discussion, Suppl. material 2).

Morphological data

Trimeresurus (*Popeia*) *popeiorum* Smith, 1937

Figs 2–4

Trimeresurus popeiorum Smith (1937).

Popeia popeorum Malhotra & Thorpe (2004a), Wallach et al. (2014), Guo et al. (2015).

Trimeresurus (*Popeia*) *popeiorum* David et al. (2011).

Trimeresurus yingjiangensis Chen, Zhang, Shi, Tang, Guo, Song & Ding, (2019), syn. nov.

Type material. ***Lectotype*:** NHMUK 72.4.17.137, a male from 'Khasi Hills' now in Meghalaya State, India. Designated by Taylor and Elbel (1958).

Additional material. 'India' one female BNHS 3613 and two males BNHS 3614 & NCBS NRC-AA-4548 Kamlang Wildlife Sanctuary, one male NCBS NRC-AA-4549 and one female NCBS NRC-AA-4534 Eaglenest Wildlife Sanctuary, Arunachal Pradesh; male MZMU 1829, MZMU 3043, female MZMU 1148 from Aizawl and male MZMU 2028 from Mamit, female MZMU 957 from Saitual, Mizoram; three males BNHS 2638, BNHS 2640, BNHS 2639 from Tindharia, Darjeeling; MZMU 3073: Pope's pitviper, female. Collector: Col.YR Singh; Location: Umroi Military Station, East Khasi Hills, Meghalaya; 'MYANMAR' ZMMU-NAP-09522 from Sagaing, Zalon Taung Mt., and ZMMU-NAP-09445 from Kachin, Indawgyi Lake.

Diagnosis. A *Trimeresurus* bearing 21 (rarely 19) dorsal scale rows at midbody with an overall bright green

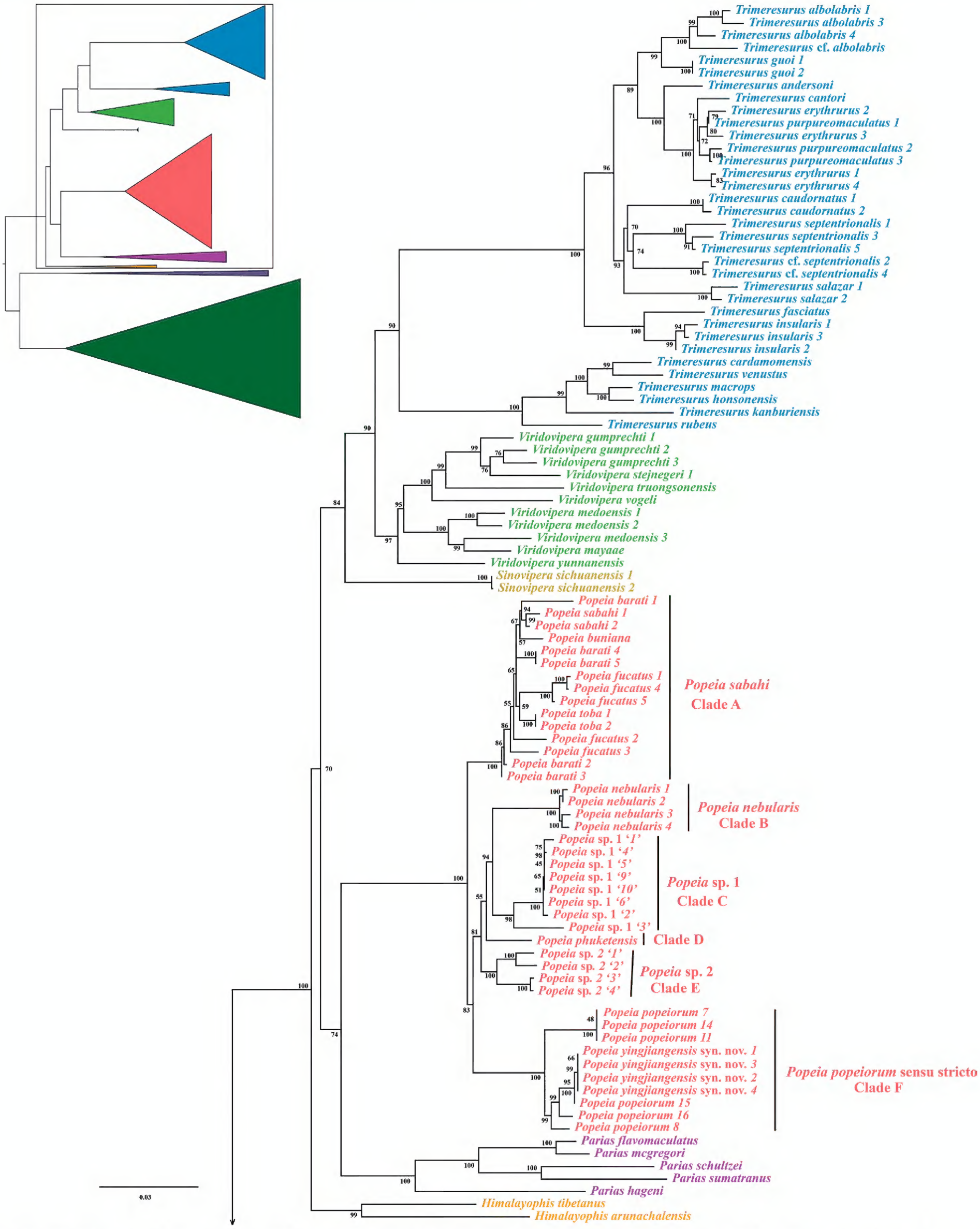

Figure 1. ML molecular phylogeny of Asian pit vipers of the genus *Trimeresurus s. l.* based on 2391bp of four mitochondrial genes (cytochrome *b*, *16S* rRNA, *12S* rRNA, and *ND4*). Numbers at nodes are clade bootstrap support recovered from 1000 non-parametric pseudo-replicates. For complete tree with outgroup see supporting files.

colour, lacking bands; males may bear a bicoloured postocular stripe and a bicoloured ventrolateral stripe is always present. SVL 414–692 mm in males, 417–710 mm in females. Hemipenis deeply forked, reaching the 25th subcaudal. Ventrals 158–170 in males, 161–169 in females; subcaudals 62–76 in males, 55–66 in females; nasal and first supralabial separate. TaL 107–194 mm in males, 75–139 mm in females; TaL/TL 0.18–0.22 in

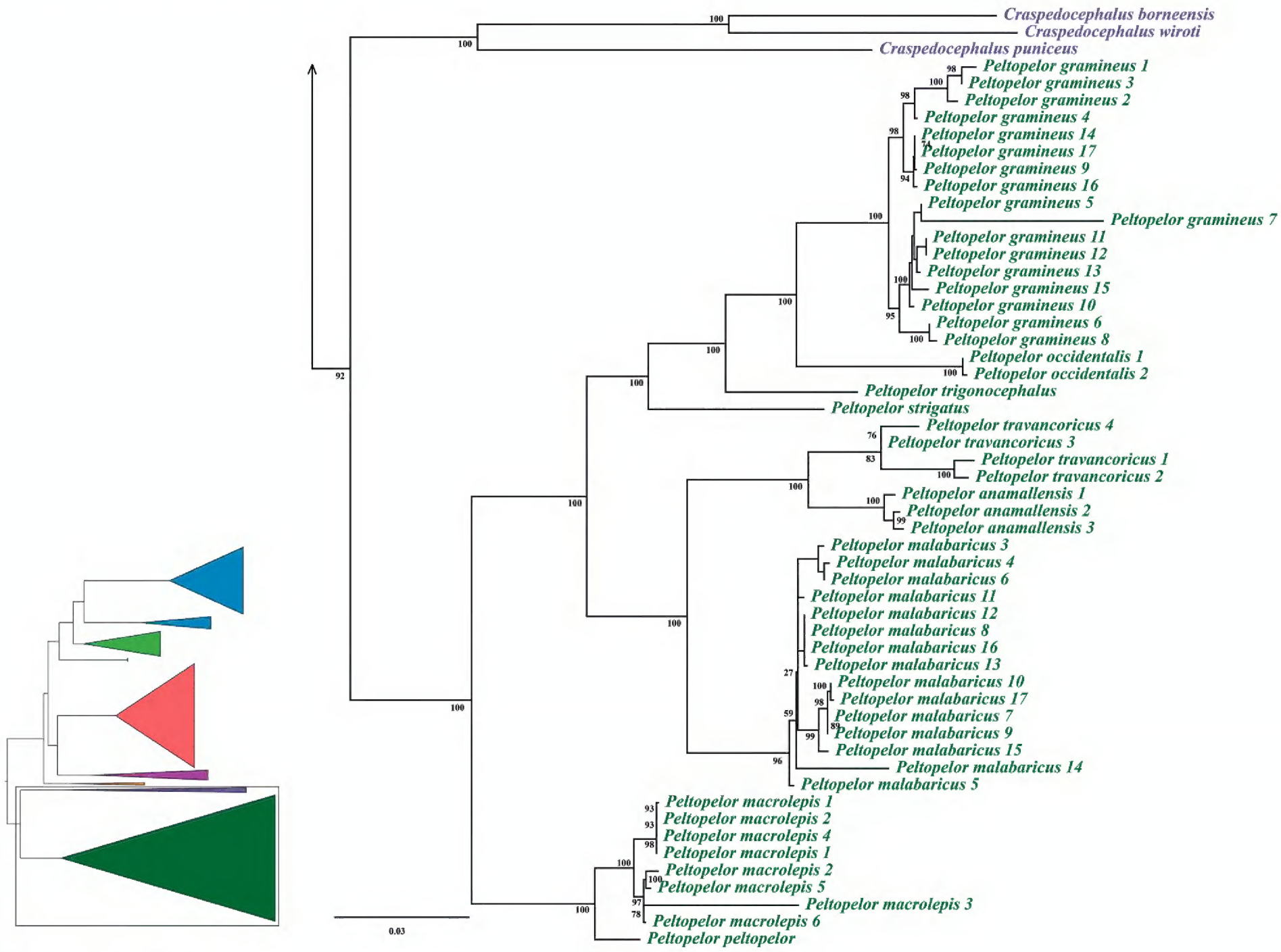

Figure 1. Continued.

Table 1. Morphological and meristic values of examined specimens of *T. popeiorum* and type series of *T. yingjiangensis* syn. nov.

Specimen	NCBS NRC-AA-4548	NRC-AA-4534	NCBS NRC-AA-4549	NCBS NRC-AA-4550	MZMU 1829	MZMU 2028	MZMU 957	MZMU 1148	DL2017070101	ZLtspynglg201801	DL201070102	DL201070103	OV2671
Sex	Male	Female	Male	Female	Male	Male	Female	Female	Male	Female	Male	Male	Male
SVL	551	550	500	460	487	600	417	485	688	710	549	675	692
TaL	120	130	115	75	114	147	80	114	176	139	148	178	194
TL	671	680	615	535	601	747	497	599	864	849	742	853	886
TaL/TL	0.18	0.19	0.19	0.14	0.19	0.20	0.16	0.19	0.20	0.16	0.20	0.21	0.22
DSR	21:21:15	23:21:15	23:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15	21:21:15
V	166 (3)	161 (3)	165 (0)	169 (1)	168	167	165	169	164	168	164	167	166
Sc	65	66	67	55	66	66	60	70	73	60	76	72	71
A	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single
SupraL	10 & 10	9 & 10	10 & 10	12 & 12	12 & 11	13/12	13/12	12 & 12	10 & 10	10 & 10	10 & 11	10 & 10	9 & 10
InfraL	11 & 12	11 & 11	13 / 12	13 & 13	10 & 10	10 & 10	10 & 10	10 & 10	12 & 12	12 & 12	12 & 12	12 & 12	12 & 12
Cephalic scales	28	27	30	31	28	28	27	–	–	–	–	–	–
Interorbital scales (least)	11	11	11	11	12	11	10	–	11	11	11	11	12
Interorbital scales (max)	17	16	16	15	15	14	15	–	–	–	–	–	–
HL	24.31	24	22.5	20.7	20.0	24.6	20.1	20.9	–	–	–	–	–
HW	17.7	21	17	16.3	15.4	17.1	13.4	13.4	22.2		18.1	19.7	20.8
Snout to neck	30.21	30	25	25.7	24.2	29.0	22.8	24.9	–	–	–	–	–
Interocular	11.7	11	11.2	9.8	10.2	12.8	9.3	9.9	–	–	–	–	–
Nares to Eye	6.34	6.7	6.4	6.5	5.7	6.5	5.2	5.6	–	–	–	–	–
Eye diameter	3.6	4	3.7	3.3	3.8	4.0	3.5	3.9	–	–	–	–	–
Eye to lip	3.5	3.9	3.7	4	3.4	3.9	3.3	3.0	–	–	–	–	–
Internarial	7	6.6	7	5.3	4.5	5.1	3.8	4.2	–	–	–	–	–
BW	13	13	11.5	13.4	9.7	11.0	7.7	10.1	–	–	–	–	–

males, 0.14–0.19 in females. Palatine with four teeth; pterygoid with eight teeth; 10–12 dentary teeth. Maxilla with one functional and 5–6 replacement fangs.

Description based on examined specimens (*n* = 19). Body long and moderately stout, SVL 414–600 mm; head triangular and elongate, head length 20–24.3 mm (HL/SVL 0.04–0.05); head width 13.4–21 mm; (HW/HL 0.64–0.88) clearly distinct from neck; distance between nostrils 3.8–7 mm; distance between preoculars 8.6–12.8 mm; distance between the tip of snout and anterior border of eye 6.6 mm; distance between nostril to eye 5.1–6.7 mm. Canthus rostralis distinct; a single large scale between the nasal and supraocular. Rostral subtriangular, slightly visible when viewed from above; nasal and first supralabial separate, wider than tall; three internasals, the outer pair of internasals larger than the one in the middle, and the one in the middle less than half the width of the outer ones. bordered by six scales on its posterior margin; two small scales separate the third supralabial from the nasal; second and third supralabial and three preoculars encompass the loreal pit; the lower preocular forms the lower margin of the loreal pit; one elongate and narrow supraocular; cephalic scales (CEP) small, irregular, subimbricate, smooth; longitudinal cephalic scales 27–31, gradually increasing in size towards the posterior part of the head; 10–11 CEP between anterior edge of the supraoculars and 14–17 at the posterior edge; occipital scales smooth; seven rows of scales between the internasals and anterior border of the supraoculars flat and irregular in their shape; the rows towards the posterior part of the head gradually show a feeble keel; temporals feebly keeled and subequal; subocular crescent shaped; 9–13 supralabials; SL1 not fused with nasal scale, 2nd much higher than 1st, 3rd highest among the supralabials; 4th widest, separated from the subocular by a single row of smooth scales and the lower loreal scale; 4th supralabial separated from the subocular by two rows of smooth scales; the remaining supralabials slightly decreasing in size posteriorly and in contact with temporal scales; 10–13 infralabials, the first pair in contact with each other; the first three pairs in contact with anterior chin shields; six pairs of chin shields, each pair in contact medially; separated from infalabials by 1–5 scale rows.

Body scalation. 19, 21 or 23 dorsal scales one head length behind the head (rarely 17); 21 dorsal scales at midbody, rarely 19; 13–15 dorsal scales one head length anterior to the vent; dorsal scales rhomboid, moderately keeled except for the first row which is smooth; 0–3 preventrals; ventrals 158–170 in males, 161–169 in females ventral scales; subcaudals 62–76 in males, 55–66 in females subcaudal scales; paired; single cloacal plate. Eye large, with VED/DEL ratio 0.85; tail short; ventrally depressed; TaL 107–194 mm in males, 75–139 mm in females; TaL/TL 0.18–0.22 in males, 0.14–0.19 in females. Tail prehensile. Hemipenis long and deeply forked at the 5–6th subcaudal, extending to the 23–25th subcaudal scales, calyculate throughout the arms of the fork, lacking spines.

Description of the skull (Fig. 2). The skull of *T. popeiorum* displays the typical shape found in other members of the genus *Trimeresurus*. The skull is well calcified except for the premaxilla which remains largely cartilaginous, and base of non-functional teeth. The braincase, or neurocranium, is composed of the prefrontals, frontals, jugals, parietal, supraoccipital, prootics, otoccipitals, basioccipital, and parabasisphenoid. The general shape of the braincase is trapezoid in dorsal view (Fig. 2b); with the main openings well visible in lateral view (Fig. 2a). Fenestra ovalis (between the otooccipital and prootic), foramina of trigeminal and facial nerves (on the prootic), and the foramen opticum (between the frontal, the parietal, and the basisphenoid) (Fig. 2a). Columella, or stapes, is well-visible attached to the fenestra ovalis (Fig. 2a).

Frontals paired, articulated with each other with a straight median suture (Fig. 2b); ventrally articulated with the parabasisphenoid, anteriorly with the prefrontals and the nasals, and posteriorly with the parietal and the jugals. Dorsally the frontals have a squared shape with slightly convex posterior and anterior margins. The parietal single, anteriorly articulated with the jugals and the frontals, ventrally with the parabasisphenoid and posteriorly with the supraoccipitals and the prootics. The parietal is a large bone which has almost a triangular shape in dorsal view (Fig. 2b) with almost straight anterior margin, short outward protruding dorsal jugal transverse processes bound by the jugals, and distinct crests seen in the lateral aspect (Fig. 2a). Parietal foramen is absent. The suppraoccipital is a single bone, anteriorly articulated with the parietal, posterolaterally with the otoccipitals, and anetrolaterally with the prootics. In dorsal aspect the anterior margin of the supraoccipital is triangularly concave (Fig. 2b); occipital crests straight, directed posterolaterally, forming a narrow angle with the sagittal crest. The prefrontals paired, subrectangular in dorsal view (Fig. 2b), ventrally articulated with the maxillae, posteriorly with the frontals; with which they are articulated via a long prefrontal caudal ramus. The prefrontals are perforated with a lacrimal foramen. The jugals (in some works referred to as postorbitals, see (Szyndlar 1984)) are paired bones, medially articulated both with the frontals and the parietal. The jugals are semi-crescent in shape with an irregular posterior margin (Fig. 2a).

The snout is composed by the nasals dorsally (Fig. 2b), premaxilla anteriorly (Fig. 2a), septomaxillae and the vomers ventrally (Fig. 2c), the latter form the bony capsule of the Jacobson's organ. Nasals paired, posteriorly articulated with the frontal, ventrally with the septomaxilla; dorsally each nasal triangular in shape, with anterior tip bearing a poorly-ossified anterior process (Fig. 2b). The dorsal margin of the posterior part of the nasal is gently rounded. The premaxilla is an unpaired, poorly ossified bone, posteriorly articulated with the vomers and the septomaxilla, with an unpaired posterior nasal process (Fig. 2b). The vomers are bubble-shaped paired bone, containing the Jacobson's organ. The vomer is dorsally articulated with the septomaxilla, and anteriorly with the premaxilla (Fig. 2c). The capsular expansion of vomer well-developed, with a wide surface directed anterodorsally. The septomaxilla is a paired bone located in the

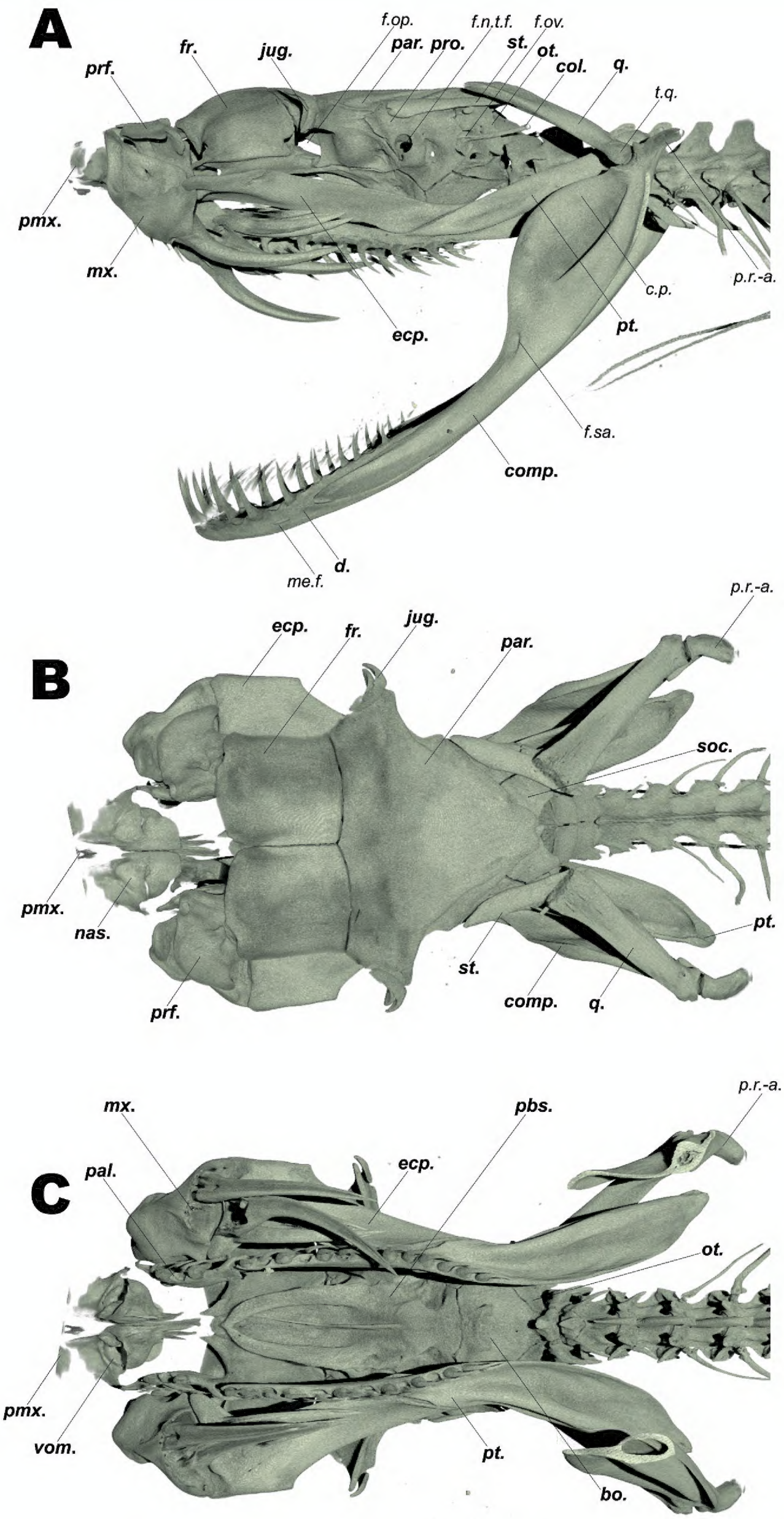

Figure 2. MicroCT scan of the skull of male *Trimeresurus popeiorum* (NCBS NRC-AA-0010) in lateral (**A**), dorsal (**B**) and ventral (**C**) aspects, showing osteological features of members of the subgenus *Popeia*. Abbreviations: bo. – basioccipital; c.p. – coronoid process; col. – columella (stapes); comp. – compound bone; d. – dentary; ecp. – ectopterygoid (transversum); f.n.t.f. – foramina of trigeminal and facial nerves; f.op. – foramen opticum; f.ov. – fenestra ovalis; f.sa. – supraangular foramen; fr. – frontal; jug. – jugal; me.f. – mental foramen; mx. – maxilla; nas. – nasal; ot. – otoccipitals; p.r.–a. – retroarticular process; pal. – palatine; par. – parietal; pbs. – parabasisphenoid; pmx. – premaxilla; prf. – prefrontal; pro. – prootics; pt. – pterygoid; q. – quadrate; soc. – supraoccipital; st. – supratemporal; t.q. – trochlea quadrati; vom. – vomer.

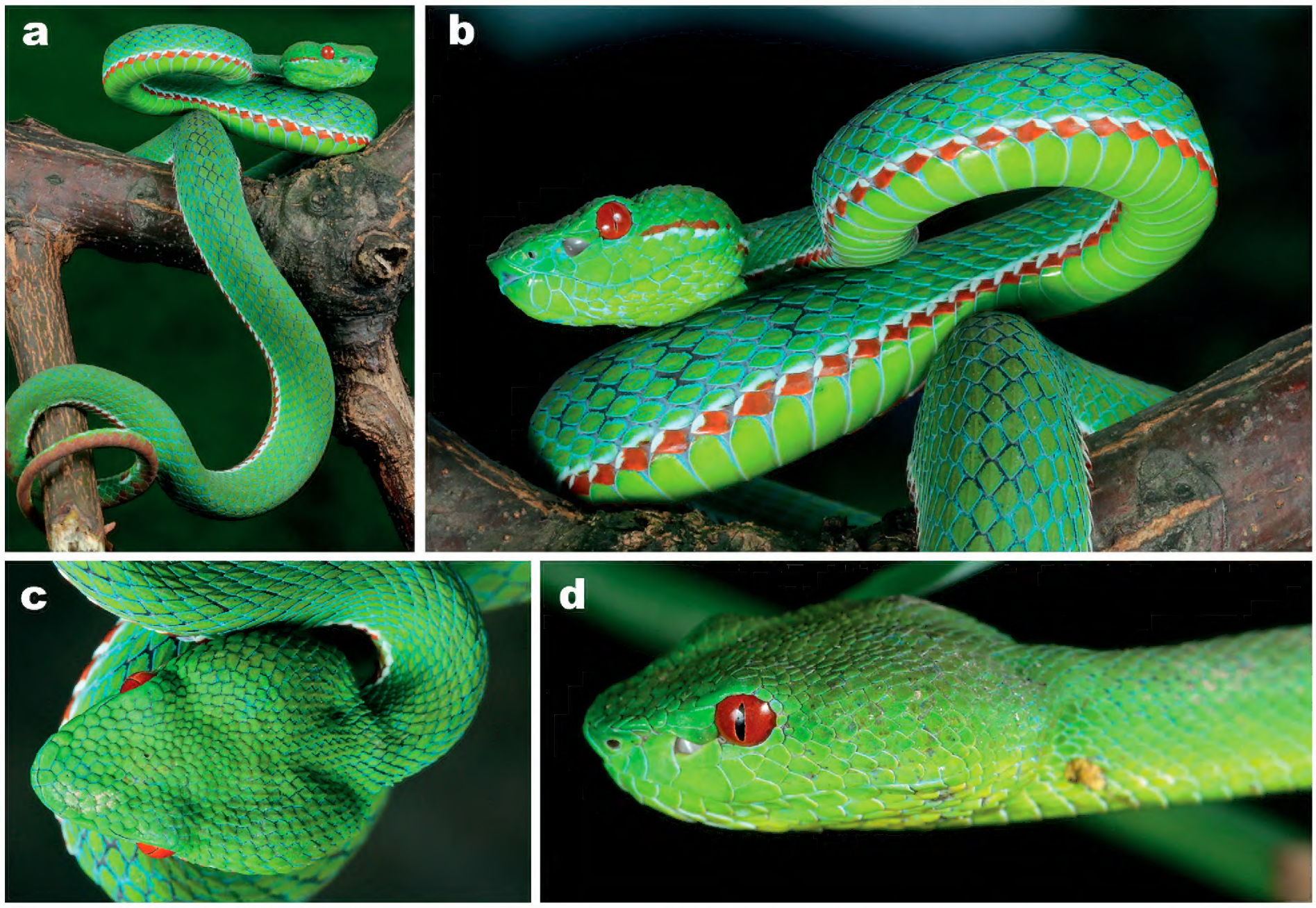

Figure 3. Images depicting colouration of *Trimeresurus popeiorum* in life, (**a**) a complete profile of a male *Trimeresurus popeiorum* from Kamlang Wildlife Sanctuary, (**b**) anterior body showing the bicolored postocular and ventrolateral stripe, (**c**) dorsal view of head, (**d**) female lacking the stripes from the same locality. Photos by Zeeshan A. Mirza.

center of the snout, articulated anteriorly with the premaxilla, dorsally with the nasals, and ventrally with the vomers; it cannot be observed in lateral or ventral aspects of the whole skull.

In ventral view (Fig. 2c) the floor of the skull is formed by the basioccipital and the parabasisphenoid. The parabasisphenoid is a single bone forming the ventral surface of the braincase, ventrally bearing a deep longitudinal cavity with a median crest. It is articulated anterodorsally with the frontals, dorsally with the parietal, posterodorsally with the prootics and posteriorly with the basioccipital. In ventral view, the bone is elongated oval-shaped, with a long and spatulate parasphenoid process; the basisphenoid crest well-developed (Fig. 2c). The basioccipital is an unpaired bone anteriorly articulated with the parabasisphenoid, anterolaterally with the prootics, and posterolaterally with the otoccipitals. The anterior margin of the basioccipital is gently concave; its' posterior margin forms the ventral surface of the foramen magnum. The prootic is a paired bone, anteriorly articulated with the parietal, posteriorly with the supraoccipital and the otoccipital, and ventrally with the parabasiphenoid. In lateral aspect, the foramen nervi trigemini is well seen in the middle part of the bone; this bone forms the anterior edge of the foramen ovale (Fig. 2a). The otoccipital is formed by the ossae opisthotici and the exoccipital (occipitale laterale). This paired bone is articulated with the supraoccipital and the prootic anteriorly, with its controlateral equivalent along a straight suture medially, and with the basioccipital ventrally. It forms the posterior edge of the foramen ovale (Fig. 2a).

The upper jaw includes the maxilla, the pterygoid, the palatine, and the ectopterygoid which form a movable connection to each other; with only the latter bone lacking teeth. Maxillae are large paired bones, kinetically articulated posterodorsally with the prefrontals and posteriorly with the ectopterygoids. Maxillae are massive bones bearing venom teeth; dorsally with a thick triangular prefrontal process forming the maxillary–prefrontal articulation, and laterally with a large fossa (Fig. 2a). Maxilla bears one functional and 5–6 replacement fangs (venom teeth); the functional fang is slightly longer than the body of the maxilla. The quadrates and the supratemporals are kinetically connected and put together the neurocranium and the lower jaw (Fig. 2a). The palatine is a paired short bone, posteriorly kinetically articulated with the pterygoids, bearing from four to five tooth alveoli with four functional teeth (Fig. 2c). The pterygoid is a large paired bone, anteriorly articulated with the palatine, posteriorly with the quadrate, and posterodorsally with the ectopterygoid. This bone is distinctly dorsoventrally bent and is slightly V-shaped in lateral aspect, bearing a pterygoid crest (Fig. 2a). Posteriorly the pterygoid bears the facet for the attachment of the ligamentum quadrato-pter-

Figure 4. Hemipenis of *T. popeiorum* MZMU 3043. Photo by Andrey Bragin.

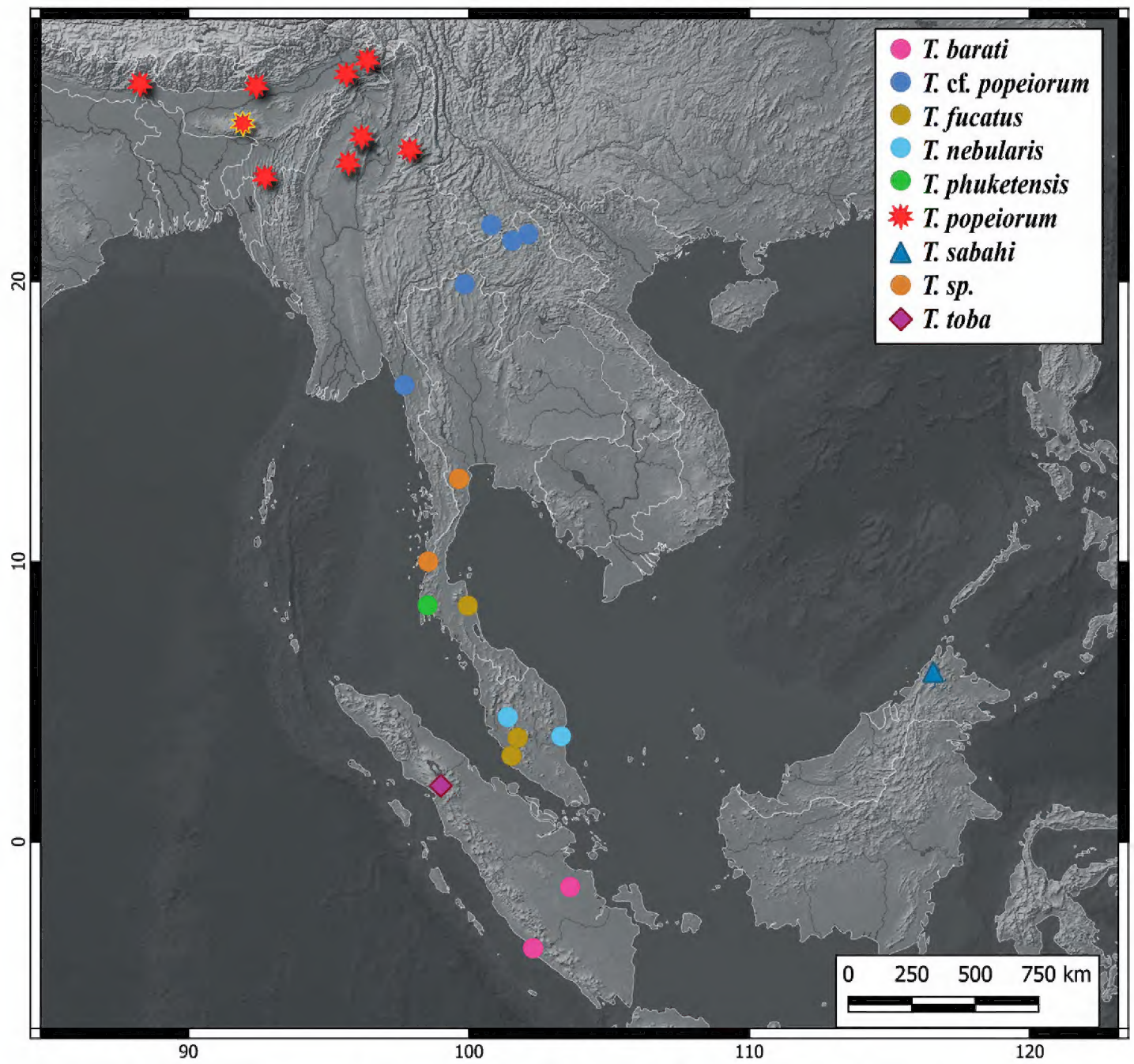

Figure 5. Map of Southeast Asia showing the distribution of members of the subgenus *Popeia*.

Table 2. Revised classification of *Trimeresurus s. l.*

David et al. (2011)	Present work
Trimeresurus (*Trimeresurus*) *albolabris* (Gray, 1842)	*Trimeresurus* (*Trimeresurus*) *albolabris* (Gray, 1842)
Trimeresurus (*Craspedocephalus*) *andalasensis* David, Vogel, Vijayakumar & Vidal, 2006	*Craspedocephalus andalasensis* (David, Vogel, Vijayakumar & Vidal, 2006)
Trimeresurus (*Trimeresurus*) *andersoni* Theobald, 1868	*Trimeresurus* (*Trimeresurus*) *andersoni* Theobald, 1868
–	*Peltopelor anamallensis* (Günther 1864)
–	*Trimeresurus* (*Himalayophis*) *arunachalensis* (Captain, Deepak, Pandit, Bhatt & Athreya, 2019)
Trimeresurus (*Craspedocephalus*) *borneensis* (Peters, 1872)	*Craspedocephalus borneensis* (Peters, 1872)
Trimeresurus (*Craspedocephalus*) *brongersmai* Hoge, 1969	*Craspedocephalus brongersmai* (Hoge, 1969)
Trimeresurus (*Trimeresurus*) *cantori* (Blyth, 1846)	*Trimeresurus* (*Trimeresurus*) *cantori* (Blyth, 1846)
–	*Trimeresurus Trimeresurus*) *calamitas* Vogel, David & Sidik 2022
Trimeresurus (*Trimeresurus*) *cardamomensis* (Malhotra, Thorpe, Mrilanili & Stuart, 2011)	*Trimeresurus* (*Trimeresurus*) *cardamomensis* (Malhotra, Thorpe, Mrilanili & Stuart, 2011)
–	*Trimeresurus* (*Trimeresurus*) *caudornatus* Chen, Ding, Vogel & Shi, 2020
–	*Trimeresurus* (*Trimeresurus*) *davidi* Chandramouli, Campbell & Vogel, 2020
Trimeresurus (*Trimeresurus*) *erythrurus* (Cantor, 1839)	*Trimeresurus* (*Trimeresurus*) *erythrurus* (Cantor, 1839)
Trimeresurus (*Trimeresurus*) *fasciatus* (Boulenger, 1896)	*Trimeresurus* (*Trimeresurus*) *fasciatus* (Boulenger, 1896)
Trimeresurus (*Parias*) *flavomaculatus* (Gray, 1842)	*Trimeresurus* (*Parias*) *flavomaculatus* (Gray, 1842)
Trimeresurus gracilis Oshima, 1920	*Trimeresurus gracilis* Oshima, 1920 *incertae sedis*
Trimeresurus (*Craspedocephalus*) *gramineus* (Shaw, 1802)	*Peltopelor gramineus* (Shaw, 1802)
Trimeresurus (*Viridovipera*) *gumprechti* David, Vogel, Pauwels & Vidal, 2002	*Trimeresurus* (*Viridovipera*) *gumprechti* (David, Vogel, Pauwels & Vidal, 2002)
–	*Trimeresurus* (*Parias*) *gunaleni* (Vogel, David & Sidik, 2014)
–	*Trimeresurus* (*Trimeresurus*) *guoi* Chen, Shi, Gao, Vogel, Song, Ding & Dai, 2020
Trimeresurus (*Parias*) *hageni* (Van Lidth de Jeude, 1886)	*Trimeresurus* (*Parias*) *hageni* (Van Lidth de Jeude, 1886)
Trimeresurus (*Trimeresurus*) *honsonensis* Grismer, Ngo & Grismer, 2008	*Trimeresurus* (*Trimeresurus*) *honsonensis* Grismer, Ngo & Grismer, 2008
Trimeresurus (*Trimeresurus*) *insularis* Kramer, 1977	*Trimeresurus* (*Trimeresurus*) *insularis* Kramer, 1977
Trimeresurus (*Trimeresurus*) *kanburiensis* Smith, 1943	*Trimeresurus* (*Trimeresurus*) *kanburiensis* Smith, 1943
–	*Trimeresurus* (*Trimeresurus*) *kirscheyi* Vogel, David & Sidik, 2022
–	*Trimeresurus* (*Trimeresurus*) *kuiburi* Sumontha, Suntrarachun, Pauwels, Pawangkhanant, Chomngam, Iamwiriyakul & Chanhome, 2021
Trimeresurus (*Trimeresurus*) *labialis* Fitzinger *in* Steindachner, 1867	*Trimeresurus* (*Trimeresurus*) *labialis* Fitzinger in Steindachner, 1867
Trimeresurus (*Peltopelor*) *macrolepis* (Beddome, 1862)	*Peltopelor macrolepis* (Beddome, 1862)
Trimeresurus (*Craspedocephalus*) *malabaricus* (Jerdon, 1854)	*Peltopelor malabaricus* (Jerdon, 1854)
Trimeresurus (*Parias*) *malcolmi* Loveridge, 1938	*Trimeresurus* (*Parias*) *malcolmi* Loveridge, 1938
Trimeresurus (*Trimeresurus*) *macrops* Kramer, 1977	*Trimeresurus* (*Trimeresurus*) *macrops* Kramer, 1977
–	*Trimeresurus* (*Viridovipera*) *mayaae* Rathee, Purkayastha, Lalremsanga, Dalal, Biakzuala, Muansanga & Mirza, 2022
Trimeresurus (*Parias*) *mcgregori* Taylor 1919	*Trimeresurus* (*Parias*) *mcgregori* Taylor, 1919
Trimeresurus (*Viridovipera*) *medoensis* Djao in Djao & Jiang, 1977	*Trimeresurus* (*Viridovipera*) *medoensis* (Zhao, 1977)
–	*Trimeresurus* (*Trimeresurus*) *mutabilis* Stoliczka, 1870
Trimeresurus (*Popeia*) *nebularis* Vogel, David & Pauwels, 2004	*Trimeresurus* (*Popeia*) *nebularis* (Vogel, David & Pauwels, 2004)
–	*Peltopelor occidentalis* (Pope & Pope, 1933)
–	*Trimeresurus* (*Popeia*) *phuketensis* (Sumontha, Kunya, Pauwels, Nitikul & Punnadee, 2011)
Trimeresurus (*Popeia*) *popeiorum* Smith, 1937	*Trimeresurus* (*Popeia*) *popeiorum* (Smith, 1937)
Trimeresurus (*Craspedocephalus*) *puniceus* (Kuhl, 1824)	*Craspedocephalus puniceus* (Kuhl, 1824)
Trimeresurus (*Trimeresurus*) *purpureomaculatus* (Gray, 1832)	*Trimeresurus* (*Trimeresurus*) *purpureomaculatus* (Gray, 1832)
Trimeresurus (*Trimeresurus*) *rubeus* (Malhotra, Thorpe, Mrilanili & Stuart, 2011)	*Trimeresurus* (*Trimeresurus*) *rubeus* (Malhotra, Thorpe, Mrilanili & Stuart, 2011)
Trimeresurus (*Popeia*) *sabahi* Regenass & Kramer, 1981	*Trimeresurus* (*Popeia*) *sabahi* (Regenass & Kramer, 1981)
–	*Trimeresurus* (*Trimeresurus*) *salazar* Mirza, Bhosale, Phansalkar, Sawant, Gowande & Patel, 2020
Trimeresurus (*Parias*) *schultzei* Griffin, 1909	*Trimeresurus* (*Parias*) *schultzei* Griffin, 1909
Trimeresurus (*Trimeresurus*) *septentrionalis* Kramer, 1977	*Trimeresurus* (*Trimeresurus*) *septentrionalis* Kramer, 1977
Trimeresurus (*Sinovipera*) *sichuanensis* (Guo & Wang, 2011)	*Trimeresurus* (*Sinovipera*) *sichuanensis* Guo & Wang, 2011
Trimeresurus (*Viridovipera*) *stejnegeri* Schmidt, 1925	*Trimeresurus* (*Viridovipera*) *stejnegeri* Schmidt, 1925
Trimeresurus (*Craspedocephalus*) *strigatus* (Gray, 1842)	*Peltopelor strigatus* (Gray, 1842)
Trimeresurus (*Parias*) *sumatranus* (Raffles, 1822)	*Trimeresurus* (*Parias*) *sumatranus* (Raffles, 1822)
Trimeresurus (*Himalayophis*) *tibetanus* Huang, 1982	*Trimeresurus* (*Himalayophis*) *tibetanus* Huang, 1982
–	*Peltopelor travancoricus* (Mallik, Srikanthan, Ganesh, Vijayakumar, Campbell, Malhotra & Shanker, 2021)
Trimeresurus (*Craspedocephalus*) *trigonocephalus* (Donndorff, 1798)	*Peltopelor trigonocephalus* (Latreille, 1801)
Trimeresurus (*Viridovipera*) *truongsonensis* Orlov, Ryabov, Thanh & Hô, 2004	*Trimeresurus* (*Viridovipera*) *truongsonensis* (Orlov, Ryabov, Thanh & Hô, 2004)
Trimeresurus (*Trimeresurus*) *venustus* Vogel, 1991	*Trimeresurus* (*Trimeresurus*) *venustus* Vogel, 1991
Trimeresurus (*Viridovipera*) *vogeli* David, Vidal & Pauwels, 2001	*Trimeresurus* (*Viridovipera*) *vogeli* (David, Vidal & Pauwels, 2001)
Trimeresurus (*Craspedocephalus*) *wiroti* Trutnau, 1981	*Craspedocephalus wiroti* (Trutnau, 1981)
Trimeresurus (*Viridovipera*) *yunnanensis* Schmidt, 1925	*Trimeresurus* (*Viridovipera*) *yunnanensis* Schmidt, 1925
–	*Peltopelor peltopelor* (Mallik, Srikanthan, Ganesh, Vijayakumar, Campbell, Malhotra & Shanker, 2021)
–	*Trimeresurus* (*Trimeresurus*) *whitteni* Vogel, David & Sidik 2022

ygoidei. The anterior half of the pterygoid is slightly curved and slender, it broadens abruptly near the middle of the bone, and flattens out, with an outward curve. The pterygoid bears from 13 to 14 tooth alveoli with eight functional teeth (Fig. 2c). The ectopterygoid, or transversum, is a paired bone which lacks teecth, and is articulated with the maxilla anteriorly and with the pterygoid posteriorly. The bone is alost spatualte in the anteior part, and gets thinner posteriorly (Fig. 2c). In dorsal view, the ectopterygoid spans out laterally, nearly half the width of the frontal (Fig. 2b). The supratemporals are paired, long and relatively thin bones, anteriorly articulated with the prootics, and posteriorly with the quadrates (Fig. 2a). The quadrate is a paired bone, anterodorsally articulated with the supratemporal and posteroventrally forming a jaw articulation with the compound bone. The trochlea quadrati is a saddle-like articular facet in the posterior part of the quadrate, kinetically articulated with the posterior portion of the compound bone (Fig. 2a).

Each mandible includes the splenial, the angular, the dentary, and the compound bone. The paired compound bone forms the posterior part of the mandible and articulates anteriorly with the dentary, anetromedially with the splenial and angular, and posteriorly is kinetically connected with the quadrate, forming the jaw articulation (Fig. 2a). The coronoid process of the compound bone is well-developed. The retroarticular process occupies the posteriormost part of the compound bone (Fig. 2a). This process is well-developed, thick, and medially curved in dorsal and ventral views (Fig. 2b, c). The saddle-like articular facet for the reception of the quadrate is located anteriorly to the retroarticular process, forming the jaw articulation. In lateral view, the supraangular foramen for the mandibular branch of the trigeminal nerve is seen on the compound bone (Fig. 2a). The angular is a paired bone, which articulates anteriorly with the splenial and the dentary, and posterolaterally is articulated with the compound bone. The splenial is a paired bone of the mandible, which is anteriorly articulated with the dentary, posteriorly with the angular, and posterolaterally with the compound bone. Both the angular and the splenial are not seen in the lateral, but only on the medial aspect of the mandible. The dentary is a paired bone, articulated with the angular and the compound bone posteriorly and with the splenial posteromedially. In lateral view, the mental foramen is visible on the dentary, it is located closer to the articulation with the compound bone than to the anterior edge of the dentary. The ventral posterior process of the dentary is slightly longer than the dorsal one (Fig. 2a). The dentary is the only tooth-bearing bone of the lower jaw, and bears from 10 to 12 functional teeth.

Colouration in life (Fig. 3). Males overall in a shade of bright green throughout, slightly lighter ventrally. Scales on the dorsum are green with cyan edges. Bicoloured postocular strip, usually red and white, runs from the posterior part of the subocular to the angle of the jaw. The line extends all the way up to the tail; the outermost dorsal scale row bears a white spot at the tip and is blood red for the rest of the part, bordered by the second row, which is white in its lower half and green on its upper half. The tail in both sexes may be rusty, red or brown. The ventrolateral aspect of the tail in males may bear a discontinuous stripe. The females lack the postocular and ventrolateral stripes. The ventral scales bear a slight yellowish tinge. The males may lack the postocular stripe. The eyes of both sexes are blood red.

Hemipenial morphology (everted organ *n* = 2, Fig. 4). *Trimeresurus popeiorum* has a hemipenis pattern corresponding fairly well to the overall pattern of the *T. macrolepis* group (Peng and Fuji 2000). Retracted organ is deeply bilobed, extends up to the 25th subcaudal and bifurcates at 6th subcaudal. The organ is bicalyculate with long, slender lobes that are twice the length of the body; capitulum restricted to sulcate and dorsal surface. The lobes are calyculate throughout; dense calyces on the distal half which transits to slightly enlarged, shallow calyces with fleshy, unadorned ridges at the base of the lobes. Sulcus spermaticus simple; the sides of the sulcus spermaticus are smooth; truncus and hemipenial base is completely nude.

Discussion

Earlier phylogenetic studies on the Asian pit vipers lacked several species in their analysis, as several were described recently. We were able to include all species of *Trimeresurus* in our analysis for which data was available, making the present work more robust. A more complete taxon sampling also corrects some of the nomenclature issues that emerged as the result of earlier analyses. Furthermore, molecular data for topotypic material of *Trimeresurus popeiorum* from northeast India sheds light on the systematics of the group and highlights the need for an integrated taxonomic approach over a morphology-based approach (Malhotra and Thorpe 2004b) to resolve cryptic pit viper species (David et al. 2011; Mirza et al. 2020; Rathee et al. 2022). The revised subgeneric nomenclature for Asian pit vipers is presented in the phylogenetic tree (Fig. 1) and summarized in Table 2, which takes the evolutionary origin of respective clades into account. Peninsular Indian and Sri-Lankan pit vipers of the subgenus *Peltopelor* represent a distinct and deeply divergent lineage from the exclusively Southeast-Asian *Craspedocephalus*; moreover, the monophyly of the clade *Peltopelor* + *Craspedocephalus* gets only a moderate level of node support in our analysis (92, see Fig. 1). Therefore we herein propose to treat *Craspedocephalus* Kuhl & van Hasselt, 1822 and *Peltopelor* Günther, 1864 as distinct genera. The analysis further suggests the presence of cryptic species across broadly distributed species like *T. septentrionalis*. The sequence of *Trimeresurus septentrionalis* (= *T. albolabris*) from Kathmandu District, Nepal generated by Malhotra and Thorpe (2000) represents the newly described *Trimeresurus salazar* which constitutes the first national record of the species from Nepal.

Trimeresurus popeiorum is described in detail as the morphological description provided by Vogel et al. (2004), which included both specimens that represent *sensu stricto* as well as other morphologically similar-looking species (*Trimeresurus* cf. *popeiorum*). *Trimeresurus popeiorum sensu stricto* is distributed across northeast India, Bhutan, northern Myanmar and the westernmost part of the Yunnan Province of China (Clade F of Fig. 5). Clades C & E of *Trimeresurus* cf. *popeiorum* potentially represent two unnamed taxa, their taxonomic assessment is in progress. Clades B and D represent *T. nebularis* and *T. phuketensis*, respectively. Samples of *Trimeresurus popeiorum sensu stricto* from India show an uncorrected p-distance of 0–0.8% for *16S* (500bp) from the sequences of the types of *T. yingjiangensis* (Suppl. material 1). Furthermore, sequences of *T. yingjiangensis* from Myanmar (Shuo et al. 2022) and those generated in the present study were found to be embedded in the clade representing *Trimeresurus popeiorum sensu stricto* (Suppl. material 2). Morphological data for *T. yingjiangensis* largely overlaps with data for *Trimeresurus popeiorum sensu stricto* and the findings are further attested by molecular data. Our molecular data leads us to conclude that *Trimeresurus yingjiangensis* Chen, Zhang, Shi, Tang, Guo, Song & Ding, 2019 represents a junior subjective synonym of *Trimeresurus popeiorum* Smith, 1937. According to the previously published data, molecular dating suggests that the subgenus *Popeia* diverged from its sister taxon ca. 15MYA (Alencar et al. 2016). The basal relationships of *Trimeresurus popeiorum sensu stricto* within the clade suggest a possible Indo-Chinese origin in the clade and dispersal into Southeast Asia; however, further studies are needed to clarify the biogeographic history of the subgenus *Popeia*.

The low genetic divergence (0–0.8% for *16S*) observed in *Trimeresurus popeiorum* across the Brahmaputra river is noteworthy as the river is known to be a barrier for gene flow for specific taxa in northeast India (Mani 1974; Pawar et al. 2006). Likely, the Brahmaputra river does not act as a biogeographic barrier for low-elevation species of pit vipers. Similar widespread distribution of *Trimeresurus salazar* across Nepal, West Bengal, Assam and Meghalaya (personal observation) supports the preceding statement. The present work also warrants the assessment of the taxonomic status of *T. popeiorum* population from Southeast Asia, especially from Thailand and Myanmar.

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Supplementary material 1

Accession numbers for sequences used in the study and sequence evolution model

Authors: Zeeshan A. Mirza, H. T. Lalremsanga, Harshal Bhosale, Gaurang Gowande, Harshil Patel, Sabira S. Idiatullina, Nikolay A. Poyarkov

Data type: table

Explanation note: Defence Research and Development Organization (DRDO, New Delhi) [sanction no. DGTM/DFTM/GIA/19-20/0422]; DST-SERB, New Delhi [DST No. EEQ/2021/000243]

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Supplementary material 2

Uncorrected sequence divergence for cyt *b* gene for selected pit vipers

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Supplementary material 3

ML phylogeny of Asian pit vipers based on cyt *b* gene

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Data type: figure

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Supplementary material 4

ML phylogeny for selected Asian pit vipers based on *16S* rRNA

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Data type: figure

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